Electron Microscopy/Atomic Force Microscopy

Principle	Name	Target/Process	Readout	Advantages	Disadvantages	References
Intercalation of psoralen and UV-mediated crosslinking of double-stranded DNA	Psoralen-EM	- Crosslinking of non-nucleosomal DNA - EM under denaturing conditions to visualize nucleosome "bubbles"	Nucleosome positioning	- Direct visualization of nucleosomal footprint - High resolution 5 to 10 bp	- Time-consuming and special equipment necessary - No sequence/ locus information unless specific locus is highly enriched	Brown et al. 2013 Hamperl et al. 2014 Brown and Boeger 2014
		- Crosslinking of replicating chromatin	DNA replication intermediates	- Direct visualization of rare replication structures (ssDNA gaps, Holiday Junctions, Fork reversal) - High resolution 5 to 10 bp		Sogo 2002 Thangavel et al. 2015
Surface sensing using a nanoscale tip, deflections are converted in a topographic image	Atomic Force Microscopy (AFM)	- Chromatin fibers and nucleosome particles	Nucleosome unfolding Nucleosome sliding	- No special sample treatment/required - High resolution in nanometer range	- Low throughput only single image about 150x150nm - Low scanning time might cause thermal drift on the sample	Miyari et al. 2011

High-Content Imaging/Fluorescent Microscopy

Principle	Name	Target/Process	Readout	Advantages	Disadvantages	References
High-content immunofluorescence microscopy against histones and histone PTMs	HiHiMap Multicolor fluorescence	- Single-cell readout/quantification across different cell cycle stages	Histone variants and Histone PTMs	- Multiplexed iterative analysis of multiple modifications - High-Throughput - Possibly useful for diagnostic applications	- Single-cell level but no single-molecule resolution - Dependent on Fluorophore stability - Application on fixed cells, no live-cell dynamics	Zane et al. 2017 Hayashi-Takanaka et al. 2020
Incorporation of thymidine analogs into newly synthesized DNA	DNA fiber stretching DNA molecular combing	- DNA chromatin fibers with active replication forks	- Multiple readouts of DNA replication dynamics: Fork speed Inter-origin distance, Fork asymmetry, Termination	- DNA fiber assays easy to implement - Monitoring a variety of replication events in the same assay - Functional analysis possible how factors affect fork dynamics *in vivo* - Megabase-sized fragments in DNA combing allows analysis of multiple replication events on same molecule	- Size of DNA fibers below typical inter-origin distance - DNA combing requires specialized equipment - No discrimination between the two newly replicating DNA strands - No sequence/ locus information unless combined with experimentally challenging DNA FISH	Czajkowsky et al. 2008 Kaykov et al. 2016 Nieminuszczy et al. 2016 Vujanovic et al. 2017 Bialic et al. 2015 Kliszczak et al. 2015 Garzón et al. 2019 Chappidi et al. 2020
Alignment of DNA molecules on a micro- and nanofabricated flowcell	Molecular curtains	- DNA-protein interactions with fluorescently labeled proteins	- Nucleosome assembly - DNA homologous recombination	- Simultaneous imaging of hundreds or thousands of aligned molecules - Detection of rare events with high statistical power	- Technically challenging due to potential uneven alignment of DNA or inefficient coverage - Need individual optimizations due to nonspecific protein adsorption	Fazio et al. 2008 Qi and Greene 2018
Fluorescence Resonance Energy Transfer (FRET)	FRET	- Distance-dependent transfer of energy from a donor fluorophore to an acceptor fluorophore	Transcription initiation and elongation	- Precise measurement of molecular distances - Accurate transcription kinetics measurements without application of mechanical forces	- No information on which probe moves - Free fluorophores can mask energy transfer - FRET pair labelling needs to be bright with the donor completely saturated by acceptor	Lee et al. 2019 Crickard et al. 2017
	Single molecule FRET (smFRET)		Effect of histone PTMs and effector proteins on nucleosome dynamics			Kilic et al. 2018 Brehove et al. 2015

Force Spectroscopy Methods

Principle	Name	Target/Process	Readout	Advantages	Disadvantages	References
Force generation upon interaction of laser beam with small particles	Optical tweezers	- Two optical beads connected by single DNA segment –proteins are at	-Transcription elongation -Nucleosome arrangement - Dynamics and frequency of pausing events	- High force and temporal resolution measurements of 0.1pN and 10^{-12} sec - Non-contact force in well-defined geometries	- Applied mechanical force could lead to artefactual behaviour - Photodamage or thermal damage of the molecules	Fazal et al 2015 Galburt et al. 2009b Galburt et al 2007
			-Histone-DNA interactions			Killian et al. 2012
Force generation by magnetic field gradient	Magnetic tweezers	- Stretch and twist of DNA molecule generated by magnetic field are recorded in real time - Immobilized single DNA molecule connected on one side to paramagnetic bead	Promoter unwinding by RNAP and the role of PIC assembly in DNA opening	- High real-time resolution measurements - Homogenous force field - Simple application on DNA stretching - Wide range of forces and lifetime of interactions from ms to hours	- Applied mechanical force could lead to misinterpretation - Lower temporal and spatial resolution compared to optical tweezers	Revyakin et al. 2012 Tomko et al 2007
			The role of PTMs on nucleosome disassembly			Simon et al. 305 2007
			Pausing events on DNA replication			Maier et al. 2000 Wuite et al. 2000

Second and Third Generation DNA Sequencing applications

Principle	Name	Target/Process	Readout	Advantages	Disadvantages	References
Single Cell Chromatin Immunoprecipitation	scChIP-seq	- Indexed chromatin labelling from individual cells	Histone PTM analysis	- Chromatin landscape of single cell	- Microfluidic setup required for sonication/library preparation - Low read depth - Strong dependence on antibody quality against target	Rotem et al. 2015
Cleave under targets and release under nuclease	CUT&RUN	- Targeted chromatin regions are cleaved by MNase and released fragments directly sequenced	- Histone PTM detection - Transcription factor - Other chromatin factors	- Limited material loss as not relying on immunoprecipitation - No need for sonication - Suitable for low-input samples - Low cost	- Adjustments needed for single-cell resolution - Strong dependence on antibody quality against target	Meers et al. 2019
Chromatin integration labeling via transposase	scChIL-seq	- Antibody-DNA conjugate (ChIL probe) mediated transposase integration of sequences that allow T7 *in situ* transcription of genomic sequences in proximity to binding site of target protein or modification	- Histone PTM detection	- Immunoprecipitation free method - Applicable to low input samples - Specific detection of chromatin regions	- Single cell resolution only achieved for abundant histone PTM targets - Bias of transposase reaction towards accessible chromatin regions - Low read depth	Harada et al. 2019
	scCUT&Tag	- Tether Protein A-Transposase to primary chromatin target and integration coupled to microfluidics device	- Histone PTM detection			Kaya-Okur et al. 2019
Barcoded and targeted chromatin release via transposase	COBATCH	- Targeted chromatin regions are cleaved by protein A-Tn5 transposase fused to specific antibodies	- Histone PTM detection			Wang et al. 2019
Bisulfite conversion of unmethylated cytosine to uracil	Bisulfite-Sequencing (BS)	- Native chromatin treated with DNA methyltransferases - PCR-amplified DNA fragments are sequenced directly or from single *E.coli* clones	- Nucleosome positioning - Endogenous DNA methylation	- Accurate DNA methylation detection - Easy to implement	- Low throughput and time- consuming workflow - Short reads - PCR amplification step can potentially lead to artifacts	Miranda et al. 2010 Kelly et al. 2012 Stadler et al. 2010
PacBio Single Molecule Real Time Sequencing (SMRT)	SMRT-BS	- Native chromatin fragments (~1.5kb) combined with bisulfite sequencing - Incorporation of fluorescent nucleotides and analysis on optical nanostructures	- Open and closed chromatin regions - Nucleosome positioning	- Sequencing in real time - Enzymatic incorporation by DNA polymerase avoids signal loss over time and longer reads with low throughput (1,5kb) - Limited artifacts by PCR and clonal selection - Accurate methylation detection	- Clone selection (even though minimal compared to BS) - Time-consuming workflow and limited throughput	Yang et al. 2015
	SAMOSA	- Chromatin fibers treated with DNA methyltransferases - Discrimination of covalently modified and not modified nucleotides based on arrival times and the fluorescence duration	- Open and closed chromatin regions - Nucleosome positioning	- Real time sequencing - Usage of DNA polymerase avoids signal degradation over time - Long reads with low throughput - No PCR artifacts	- No standard bioinformatic pipeline available - Optimization of methylation conditions - Error-rate unclear when multiple methylation/modified bases present	Abdulhay et al. 2020
	Fiber-seq					Stergachis et al. 2020
Oxford Nanopore Sequencing	MeSMLR-seq	- Chromatin treated by DNA methyltransferases - Discrimination between 4 canonical and modified DNA bases passing through a biological pore based on ionic current	- Open and closed chromatin regions - Nucleosome positioning	- Real time analysis on personal computer - Long reads up to megabase size - No PCR artifacts - Simple and rapid experimental set-up - Direct detection of modified nucleotides without chemical modificatons	- No standard bioinformatic pipeline available - Optimization of DNA methylation conditions essential - Error-rate unclear when multiple methylation/modified bases present	Wang et al. 2019
	SMAC-seq					Shipony et al. 2020
	D-Nascent	- Detection of BrdU nucleotides based on ionic current as fifth DNA base to reveal reads from actively replicated DNA	- Fork progression - Pausing events on DNA replication			Hennion et al. 2018 Müller et al. 2019 Hennion et al 2020 Georgieva et al. 2020